

ESSAY

Why Some People Prefer Pickle Juice: The Research of Dr. Richard P. Lifton

Peter M. Gayed

Yale University School of Medicine

In this essay, the author interviews Dr. Richard P. Lifton and examines the use of genomics throughout his studies on the molecular pathophysiology of hypertensive and hypotensive diseases. To date, Dr. Lifton has identified 10 different gene mutations that result in severe hypertension and another 10 that result in severe hypotension.

Dr. Richard Lifton has seen patients who crave nothing more than pickle juice. “Another favorite snack is a salted lemon,” reports Lifton, a Howard Hughes Medical Investigator and chairman of the Department of Genetics at Yale. His professional career has been devoted to understanding the complexities governing salt retention and blood pressure, in which dietary preferences play a role. So far, Lifton counts 10 different genes and gene mutations that result in severe hypertension and another 10 that result in severe hypotension.

“Examining the extremes has revealed a lot about the way our bodies regulate blood pressure,” explains Lifton. Remarkably, these genes and their products all converge on a single, powerful regulator of blood pressure: renal salt retention. When the kidneys are unable to excrete salt, water is retained in the blood. “When too much water is retained,” Lifton says, “blood pressure goes up.” The converse is also true: When too much salt is excreted in the urine, water is lost, and the result is hypotension.

The chronically hypotensive body tries to compensate in many ways, and one of those ways is manifested by a craving for salty products — and what better than pickle juice?

The idea that the body might respond to hypotension by increasing its salt intake made good sense and was supported by reports that the most hypotensive members of an affected family also ingested the most salt. What remained unclear to scientists and clinicians, however, was the primary organ responsible for salt retention and salt wasting. “To address this problem,” Lifton wrote in a 2002 review, “our group has taken a genetic approach to the understanding of hypertension in humans, using the paradigm of positional cloning,” which relies on knowing the approximate location of a gene thought to be involved with a particular disease phenotype [1].

During the lab’s many chromosomal walks, Lifton and his colleagues have revealed the molecular pathophysiology of an impressive number of diseases affecting salt

†Abbreviations: PHA, pseudohypoaldosteronism; ENaC, epithelial sodium channel; GRA, glucocorticoid-remediable aldosteronism; SSCP, single-strand conformational polymorphism; MR, mineralocorticoid receptor.

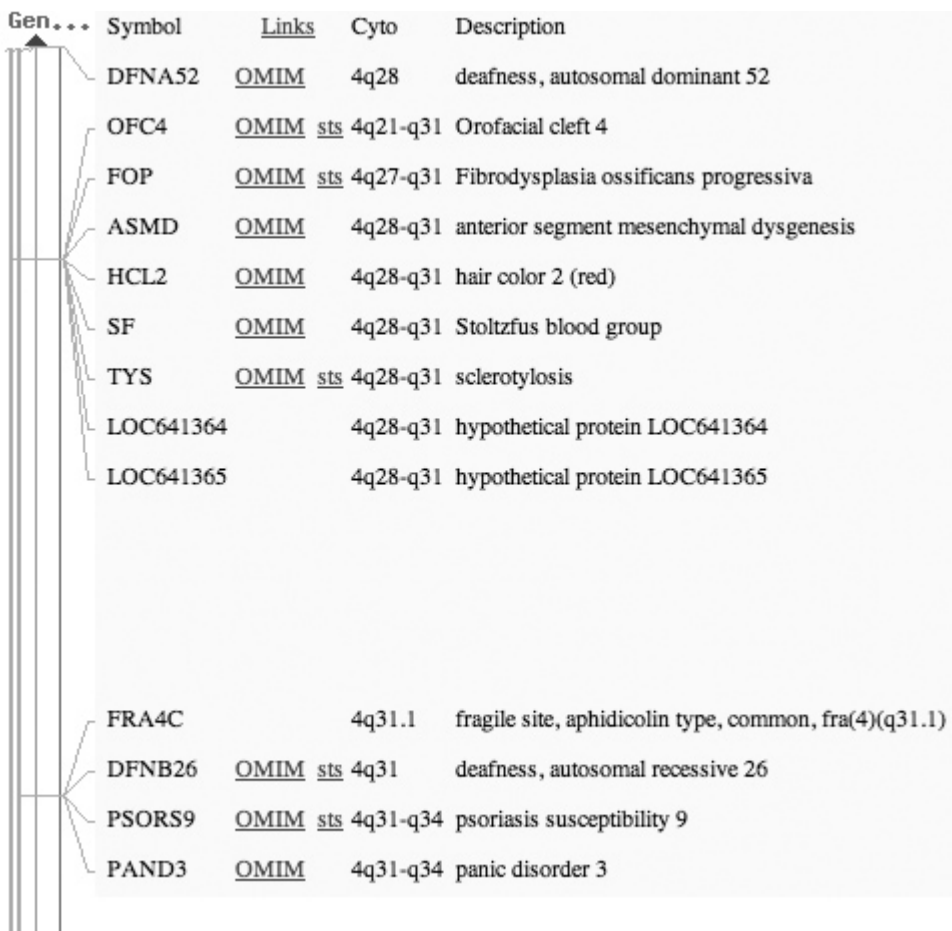


Figure 1. The accepted genetic map for the region associated with pseudohypoaldosteronism type 1. (From the NCBI Map Viewer, chromosomal region 4q31.1)

regulation. Among them are two forms of type I pseudohypoaldosteronism, Gitelman’s syndrome, Bartter’s syndrome, glucocorticoid-remediable aldosteronism, Liddle syndrome, and pregnancy-induced hypertension (the mechanism of which, ironically, was characterized while studying an adolescent boy who suffered from significantly elevated blood pressure). The last three of these diseases all cause hypertension, and until recently, physicians could do little more than haphazardly prescribe any number of 70 antihypertensive drugs. Using genomic methods and data, however, Lifton and his colleagues more precisely have described the distinct and varied genetic causes of hypertension in several diseases.

Previous to 1980, it only would have been possible to identify disease genes if such

genes encoded protein products that could be purified [2]. Once isolated, fragments of proteins were subjected to amino acid sequencing. Scientists then could construct several oligonucleotides that corresponded to all possible codon permutations of the amino acid sequence. The batch of synthesized oligonucleotides then could be used to screen a library of expressed genes (cDNA) in hopes of identifying the gene of interest.

Now scientists like Lifton use markers unique to a monogenic disease to locate, clone, and study a disease gene. Genetic markers themselves first can be identified by collecting tissue samples from healthy and afflicted family members and then analyzing those samples for specific sequence patterns, such as single nucleotide polymorphisms.

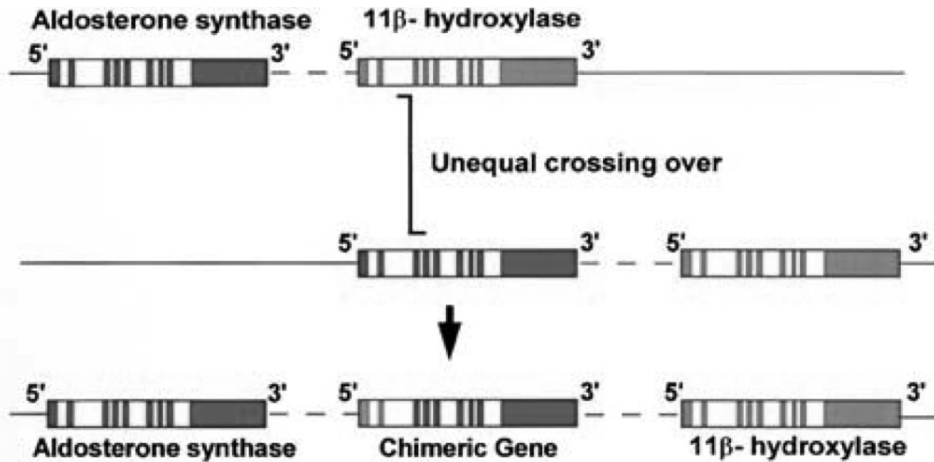


Figure 2. Schematic description of the gene fusion event which results in the constitutive production of the hormone aldosterone. (Courtesy of Dr. Richard P. Lifton)

Markers in proximity to a disease gene are inherited with it, while distant markers show weak or no co-inheritance with the gene. Genetic maps display this information as representations of chromosomes, their genes, and the relative distances between specific gene loci (Figure 1). Presumably, affected members of a family share the same mutation and, thus, exhibit a shared genetic pattern that can be elucidated by sequencing methods. Using sequenced DNA fragments, geneticists can search the Human Genome Project to identify and home in on candidate regions likely to harbor the disease gene of interest.

In 1996, for example, the Lifton lab published a study in which it used the known sequence of a suspected sodium channel to identify genotypic variations among healthy individuals and individuals suffering from pseudohypoaldosteronism (PHA^r) type 1. PHA is a potentially fatal disease marked by profound hypotension despite abnormally high levels of circulating aldosterone, a steroid hormone that normally *increases* blood pressure [6]. As Lifton predicted, affected patients were found to be homozygous for a mutation that results in the absence of the epithelial sodium channel (ENaC), the primary channel by which the kidney reabsorbs sodium.

In another study, Lifton examined glucocorticoid-remediable aldosteronism (GRA), which led to the discovery of a novel protein

caused by the fusion of two separate genes, one encoding aldosterone synthase and the other encoding 11-beta hydroxylase (Figure 2). In GRA, the resultant chimeric protein exhibits constitutive aldosterone synthase activity and, consequently, causes overproduction of aldosterone, leading to hypertension [3]. Lifton's group used a kindred to establish complete linkage to the 11-beta hydroxylase locus. Again exploiting known genomic data, the group constructed various gene fragments to demonstrate the existence of the fusion protein by single-strand conformational polymorphism (SSCP). In SSCP, one strand of DNA is amplified and run on a gel; differing secondary structures, due to various mutations, cause different bands to appear, which subsequently can be sequenced.

In contrast to GRA, Liddle syndrome is marked clinically by hypertension in the *absence* of elevated aldosterone levels. In its study of Liddle syndrome, the Lifton group mapped the phenotype to the gamma-ENaC gene subunit and sequenced it to confirm a suspected premature-stop mutation, distinct from the mutation observed in PHA patients [4]. The group analyzed a series of missense mutations in an oocyte system to confirm the role of this mutation in the pathogenesis of Liddle syndrome. The mutation, the group explains, prevents the channel's clearance from the cell surface by causing truncation of the signal sequence that allows for ubiquitin-

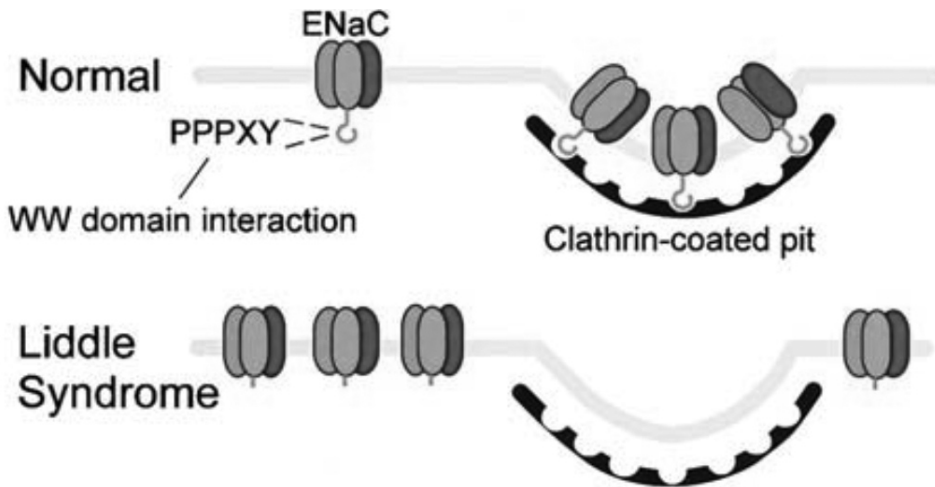


Figure 3. Luminal surface of distal nephron in healthy individual (top half) and patient with Liddle syndrome (bottom half). In Liddle syndrome, the signal sequence known as PPPXY is lost, which impairs the normal clearance of ENaC via clathrin-coated endocytosis. (Courtesy of Dr. Richard P. Lifton)

mediated degradation (Figure 3). The prolonged presence of ENaC results in enhanced sodium reabsorption and hypertension.

One of the more recent and surprising discoveries of the Lifton lab, however, came in 2002 when a resequencing of genes involved in the salt-retaining pathway revealed a mutation in the mineralocorticoid receptor (MR) of a 15-year-old hypertensive boy [5]. Normally, binding of aldosterone to MR causes increased salt reabsorption by enhancing the activity of ENaC. However, the mutation, which replaces a single serine residue with leucine, causes the receptor to be partially activated in the absence of aldosterone. More interestingly, however, is that the mutation enhances the receptor's sensitivity to progesterone and completely inverts the role the steroid has on the receptor; that is, in the presence of the mutation, progesterone, which usually acts as an antagonist, becomes a potent *agonist*. Considering that progesterone levels can increase 100-fold during gestation, this finding suggests the MR mutation also may be responsible for the severe pregnancy-induced hypertension observed in some women. Indeed, in Lifton's study, two female carriers of the mutant allele had multiple hypertensive complications during each of their pregnancies [6].

Lifton also has demonstrated that salt-wasting defects, such as those seen in Gitel-

man's syndrome, result in lowered blood pressure. In a 2001 investigation, the Lifton lab studied individuals who carried zero, one, or two copies of a mutant gene encoding a sodium-chloride cotransporter in order to ascertain its influence on blood pressure [6]. Mutations were identified using SSCP. The group found that homozygous mutant individuals had diastolic blood pressures about 8 mm Hg lower than their wild-type and heterozygous kindred. Although no significant blood pressure differences existed between the heterozygous group and the wild-type group, members of the former consumed significantly more salt in their diet, which Lifton explains as a compensatory dietary preference counterbalances the mild salt-wasting defect.

For as much as Lifton has accomplished, the problem of hypertension remains a difficult one because the majority of hypertensive patients do not have a mutation that leads to profound physiological defects. Rather, it is more often the case these patients are afflicted by subtle and varied genotypic patterns that give rise to a set of heterogeneous disease states we have grossly named "chronic hypertension."

"If there are many genes that influence [chronic hypertension] by a common path-

way,” Lifton says, “one could explain variation of disease risk within the population.” Lifton’s use of genomics has changed the very way he foresees geneticists applying the technology: “These [variations] can only be found by resequencing genes, pathways, or genomes. In coming years, we expect technologies for resequencing the human genome to plummet. Initial efforts will instead focus on resequencing a small number of high priority genes in thousands of subjects. This will evolve next to resequencing all the genes and conserved elements, which constitute only a few percent of the genome. Finally, we will begin resequencing entire genomes in large cohorts.”

Of course, Lifton is not merely forecasting the future of genomics — he is already living it. “We have recently completed such a resequencing study of salt-handling genes in the Framingham Heart Study cohort,” he announces. The results, no doubt, will lead to valuable discoveries. Until Lifton figures things out, however, some of us will have to go on drinking pickle juice.

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